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In vitro effect of *Bartonella birtlesii* on mouse red cell viability

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INTRODUCTION

The genus *Bartonella* comprises a unique group of facultative intracellular bacteria that use arthropod transmission as a mammalian parasitism strategy. Common traits of *Bartonellae* are haemotropic infections in their mammal reservoir hosts without causing immediate detriment to the host. When they are accidentally introduced into the wrong host or into immunocompromised individuals, infection results in acute clinical manifestations. After intravenous inoculation and before stable blood colonisation, *Bartonella* sp. first infect a primary niche, which could be, according to current opinion, based on in vivo clinical manifestation and on in vitro evidence, the vascular endothelial cells [1]. From this primary niche, the bacteria then colonise the bloodstream. *Bartonella*-mediated bacteraemia has been investigated using the *B. tribocorum*-rat infection model [2]. This study demonstrated that *Bartonella* associated with mature erythrocytes entered within erythrocytes and replicated until reaching about eight bacteria per cell. The number of intracellular bacteria remained constant for the remaining lifespan of the erythrocytes.

Detailed investigation of the erythrocyte invasion process requires a suitable in vitro model for erythrocyte infection. In this study, we evaluated the effect of the mouse-adapted *Bartonella birtlesii* on viability of Balb/C mouse erythrocytes maintained in different culture media, with the aim of establishing an appropriate medium to study in vitro red cell infection.

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No conflicts of interest declared.

MATERIAL AND METHODS

Bacterial strains and growth conditions

Bartonella birtlesii (IBS 135^T, CIP 106691^T) was grown for 5 days on Columbia agar containing 5% defibrinated sheep blood (CBA) in a humidified atmosphere with 5% CO_2 at 35°C.

Animal housing

Balb/C mice provided by Charles River Laboratories (Lyon, France) were housed in an animal facility (two mice/cage). All animals did not exhibit any signs of disease during the experiments. They were allowed to acclimatise to the facility and the diet for at least 5 days prior to blood being taken.

In vitro incubation of *B. birtlesii* with murine erythrocytes

Erythrocytes from the peripheral blood of mice were isolated and purified by Ficoll gradient centrifugation. After washing in PBS, they were maintained at 2.10^8/mL in different medium (F12, RPMI, brain heart infusion (BHI) and Schneider), all supplemented with 10% fetal calf serum, glutamine (2 mM), sodium pyruvate (1 mM), Hepes (0.1 mM), Histidine (257 mM), Hematin/Histidine (0.1 mg/ml), non-essential amino acid 1× concentrate (Gibco, France) [3]. After 5 days of culture on CBA plates, *B. birtlesii* were harvested, washed, suspended in PBS, and added to erythrocytes at a multiplicity of infection varying from 0.01 to 10 and incubated at 35°C in 5% CO_2 for 3 days.

Erythrocyte lysis evaluation

After 3 days of incubation, 100 microlitres of the mixture erythrocytes/*B. birtlesii* were washed in PBS and intact erythrocytes were counted under a microscope. Results are given in % of intact erythrocytes at day 3 (=100 × number of erythrocytes/mL at day 3/2×10^8).

RESULTS

Effect of *B. birtlesii* on red cell viability

First, with the aim of identifying the best medium to insure red cell viability, we evaluated red cell lysis after 3 days of culture in complete F12, RPMI, Schneider and BHI media (Fig. 1). Complete F12 and RPMI media allowed the survival of

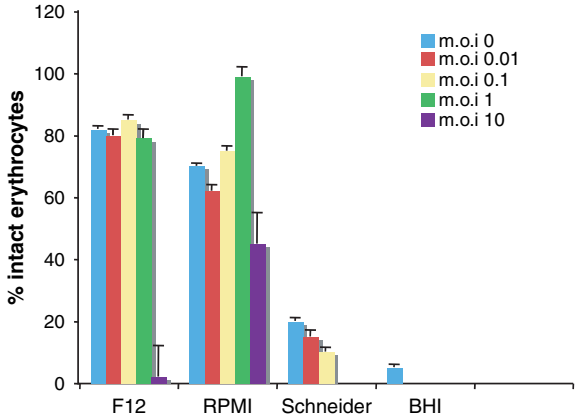

Fig. 1. Effect of *Bartonella birltesii* on the viability of mouse erythrocytes incubated in different media for 3 days.

respectively 80 (±1.5) and 70 (±2) % of erythrocytes ($n > 10$). In contrast, 3 days of incubation in either complete Schneider or BHI media induced massive lyses of erythrocytes (Fig. 1).

The effect of *B. birtlesii* on the lysis of mouse erythrocytes after 3 days of incubation was then evaluated (Fig. 1). When using complete F12 medium, *B. birtlesii* at m.o.i. of 0.01, 0.1 and 1 had no effect on cell viability. For m.o.i. of 10, 2% of erythrocytes were still intact after 3 days of incubation. With complete RPMI medium, no significant effect on cell viability was observed (compared with red cells maintained without bacteria in RPMI). In parallel we quantified bacteria at day 3 by counting unit forming colonies/mL. After 3 days of incubation, more than half of the inoculum was rescued in both complete F12 and RPMI media.

DISCUSSION

Despite a good knowledge of the dynamics of *Bartonella* parasitism of erythrocytes, very little is known about the molecular mechanisms that underlie this exploitation, due in part to the lack of an adequate in vitro red cell infection model.

In this study we defined either RPMI or F12 as adequate to maintain mouse erythrocytes alive for up to 3 days, at 35°C in 5% CO_2 atmosphere. Moreover, we showed that at a multiplicity of infection of 1 or under, *B. birtlesii* was not toxic for mouse red cells in both medium. At a multiplicity of infection of 10 or above, *B. birtlesii* induced a massive lysis of erythrocytes maintained in F12 medium and a moderate lysis when red cells are incubated in RPMI. We suggest using either complete RPMI or F12 medium with bacteria at multiplicity of infection under 10 to study red cell infection.

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